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Early Detection of Neurodegeneration in Brain Ischemia by Manganese-enhanced MRI

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Abstract—This study aims to employ *in vivo* manganese-enhanced MRI (MEMRI) to detect neurodegenerative changes in two models of brain ischemia, photothrombotic cortical injury (PCI) and transient middle cerebral artery occlusion (MCAO) in rodents. After systemic Mn^{2+} injection to both ischemic models, a close pattern of T1-weighted hyperintensity was observed throughout different brain regions in comparison to the distribution of GFAP, MnSOD and GS immunoreactivities, whereby conventional MRI could hardly detect such. In addition, the infarct volumes in the posterior parts of the brain had significantly reduced after Mn^{2+} injection to the MCAO model. It is suggested that exogenous Mn^{2+} injection may provide enhanced MEMRI detection of oxidative stress and gliosis early after brain ischemia. Manganese may also mediate infarctions at remote brain regions in transient focal cerebral ischemia before delayed secondary damage takes place.

I. INTRODUCTION

MANGANESE (Mn) is an essential constituent of two important metalloproteins involved in the pathophysiology of cerebral hypoxia and ischemia. Mn binds to the mitochondrial Mn-superoxide dismutase (MnSOD) enzyme, which acts against cellular oxidative stress. It also binds to glutamine synthetase (GS), which is a glial specific enzyme for regulating extracellular glutamate and reducing glutamate excitotoxicity. In experimental animal models of stroke, delayed hyperintensity was found in T1-weighted images (T1WI) due to MnSOD accumulation at the ischemic core [1], whereas upon exogenous Mn^{2+} injection to neonatal rat models of hypoxic-ischemic encephalopathy, upregulation of MnSOD and GS activities

was observed, leading to Mn-enhanced MRI (MEMRI) detection of lesions undetectable by other MR modalities [2, 3]. Given a previous study indicating a transient increase of MnSOD in remote brain areas after focal photothrombotic cortical injury (PCI) [4], a relatively non-invasive and reproducible model for stroke, this study aims to employ *in vivo* MEMRI to detect transient changes in adult rat model of PCI in different brain regions. Further, given the previous reports on the role of manganese as an antioxidant after manganese chloride administration to animals [5, 6], we attempted to test the feasibility of mediating remote infarction by delayed administration of manganese after transient middle cerebral artery occlusion (MCAO), in addition to the reproducibility of enhanced MEMRI detection of neurodegeneration in brain ischemia to different strains of rodents.

II. MATERIALS AND METHODS

A. Animal Preparation

Adult Sprague-Dawley rats (200-250 g, N=8) and adult C57BL/6N mice (18-22 g, N=8) were subjected to PCI in the motor cortex center [4] and 30 min-transient MCAO [7], respectively, and were divided into 4 groups (N=4 each). Two days after surgery, PCI rats in Group 1 and MCAO mice in Group 3 were administered with an intraperitoneal injection of $MnCl_2$ solution (45 mg/kg, 100 mM), while the other animals in Groups 2 and 4 received no injection. MRI was performed to the PCI animals at 3, 7, 14 and 21 days after surgery, whereby 2 rats from each group were sacrificed for histology after MR examinations at Day 7. MEMRI was also performed on 3 additional normal rats before, 1 day and 5 days after Mn^{2+} injection to compare with the PCI groups at the corresponding time points with and without Mn^{2+} administration. For MCAO animals, MRI was performed at 3, 7, 10, 14, 21 and 150 days after surgery, whereby 2 mice from each group were sacrificed for histology after MR examinations at Day 10.

B. MRI Protocols

All MRI measurements were acquired on a 7T Bruker scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), the animals were kept warm under circulating water at 37 °C, and were imaged using a receive-only surface coil for the PCI model, and a mouse brain quadrature resonator for the MCAO model. 2D T1-weighted

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(T1W) RARE sequence was acquired with TR/TE = 400/7.5 ms, RARE factor = 4, and matrix resolution = 256x256. For PCI model, FOV = 3.2 x 3.2 cm², slice thickness = 1 mm, and NEX = 16. For MCAO model, FOV = 2.0 x 2.0 cm², slice thickness = 0.7 mm, and NEX = 20. T2-weighted imaging (T2WI) was performed under the same dimensions with TR/TE = 6500/120 ms, RARE factor = 12, and NEX = 2 and 4 respectively for PCI and MCAO. Spin-echo EPI diffusion weighted images (DWI) were acquired with TR/TE = 3000/28 ms, matrix resolution = 128x128, NEX = 4, b = 0 and 100 s/mm², number of shots = 4 and 30 diffusion directions. FOV = 3.2 x 3.2 cm² for PCI model and 3.0 x 3.0 cm² for MCAO model.

C. Data Analysis

The T1W signal intensities at the hypointense ischemic core and peri-infarct areas 0.5 mm to the core border were measured in both ischemic models using ImageJ v1.38x (Wayne Rasband, NIH, USA). The total brain volumes, and the total infarct volumes indicated by T2W hyperintensity were also measured in the ipsilesional side of the brain. Mean values were compared using two-tailed Student's t-tests, and the values along the time course were compared using ANOVA. Results were considered to be significantly different when $p < 0.05$.

D. Histology

After MR examinations, the animals were transcardially perfused with 4% paraformaldehyde. The brains were then removed, cut into 10 μ m sections, and immunostained for glial fibrillary acidic protein (GFAP), manganese superoxide dismutase (MnSOD) and glutamine synthetase (GS), which are markers for gliosis, oxidative stress and glutamate excitotoxicity, respectively.

III. RESULTS

A. Enhanced MEMRI detection in PCI model

When no Mn²⁺ was applied to the PCI animals in Group 2, the dynamic changes of T2WI and DWI abnormalities generally followed as previously described [8]. At Day 3 after photothrombosis, no T1W signal enhancement was observed in the PCI model at the ipsilesional perilesional rim ($p > 0.05$) as in Figure 1. Yet at Day 7 when the MnSOD activity was supposed to peak in the peri-infarct areas [4], mild T1W signal enhancement was observed at the perilesional rim upon windowing. However, such increase was not statistically significant when compared to the same regions in the normal rats before Mn²⁺ injection ($p > 0.05$), even though MnSOD and GS immunoreactivities were observed in the ipsilesional cortex as in Figure 2. The apparent T1W signals then dropped as time went by.

After Mn²⁺ injection, T1W hyperintensity was observed at the perilesional rims in all animals. At Day 3 and Day 7 after photothrombosis, animals in Group 1 gave a significant increase in signal intensities at the rims compared to the

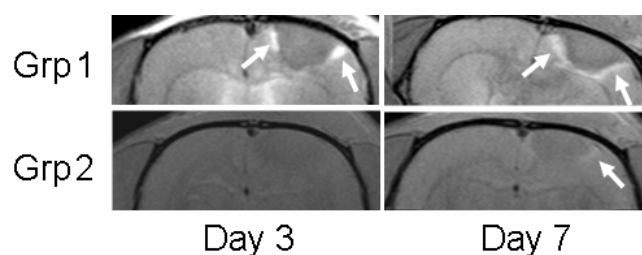


Fig. 1. Typical T1WI at the center of ischemic core at 3 and 7 days after photothrombosis, showing hyperintensity in the perilesional rims (arrows) with (Group 1) and without (Group 2) Mn²⁺ injection. Hyperintensity was observed in the perilesional rims in Group 2 at Day 7 but was less obvious.

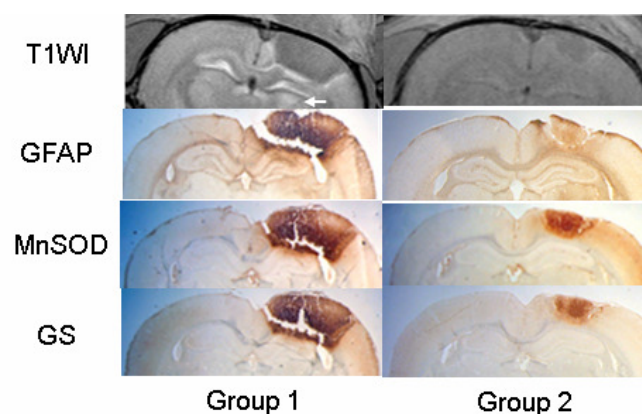


Fig. 2. T1WI at the ischemic core at Day 7 following photothrombosis with (Group 1) and without (Group 2) Mn²⁺ injection, and the corresponding GFAP, MnSOD and GS immunohistochemical stains at Day 7. Note also the enhancement in the ipsilesional thalamus in Group 1 (arrow).

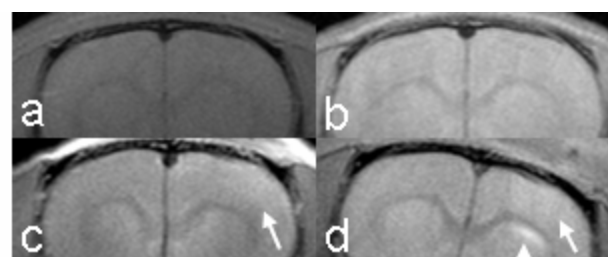


Fig. 3. (Top) Typical T1WI of a normal rat (a) before and (b) 24 hours after Mn²⁺ injection. (Bottom) Typical T1WI at about 2 mm anterior to the ischemic core at (c) 3 days and (d) 7 days after photothrombosis, showing hyperintensity in the ipsilesional cortex (arrows) after Mn²⁺ injection at day 2 in Group 1.

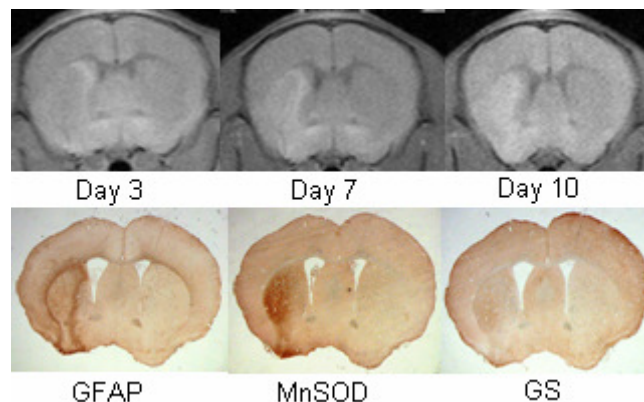


Fig. 4. Mn-enhanced MRI of the striatum at 3, 7 and 10 days following MCAO in Group 3, and the corresponding GFAP, MnSOD and GS immunohistochemical stains at Day 10.

same location in both the Group 2 animals without Mn^{2+} injection ($p<0.05$), and the normal rats after Mn^{2+} administration ($p<0.05$). Enhancement could also be found in the ipsilesional cortex distinct from the ischemic core in Figure 3 and occasionally the subcortical regions in Figures 2 and 3 compared to the normal brains after Mn^{2+} administration.

B. Enhanced MEMRI detection in MCAO model

In the ipsilesional dorsolateral striatum, when no Mn^{2+} was applied in the MCAO mice in Group 4, signal intensity increased in T1WI (ANOVA, $p<0.01$) and decreased in T2WI (ANOVA, $p<0.01$) from Day 3 to Day 21 after MCAO. These were in agreement with previous studies suggestive of delayed ischemic striatal neurodegeneration [1] and inflammatory responses [9]. The diffusion trace values had also dropped in the corresponding hyperintense regions in T2WI, indicative of the presence of oedema. At Day 3, a significant increase in signal intensity at the perilesional rim was observed in T1WI compared to the ischemic core in Group 3 after Mn^{2+} injection for 24 hours (paired t-test, $p<0.05$) but not Group 4 ($p=0.18$), while the Mn-enhanced MRI pattern at Day 10 was in good colocalization with GFAP, MnSOD and GS immunostainings in Figure 4.

C. Total infarct volumes in PCI and MCAO models

The infarct volumes indicated by hyperintense regions in T2WI dropped significantly in both models as time went by. The total infarcted volumes in ipsilesional forebrain were not different between the two MCAO groups as in Figure 5. However, in the brain regions remote to the ischemic core, T2W hyperintensity was observed in the hippocampus, thalamus, midbrain and superior and inferior colliculi in all mice in Group 4, while mild infarction could only be found in the thalamus of one of the mice in Group 3. As shown in Figure 6, total infarcted volumes in the posterior parts of the ipsilesional brain had significantly reduced after Mn^{2+} injection. Note that at 150 days after MCAO, shrinkage of the midbrain and the superior and inferior colliculi was observed in all the 2 remaining animals in Group 4 but not Group 3 as shown in Figure 7. No significant difference was observed in the total infarct volumes between Group 1 and Group 2 in the PCI model.

IV. DISCUSSIONS

In the present study we demonstrated the early detection of neurodegeneration in brain ischemia by MEMRI in both PCI and MCAO models of different animal strains. These patterns were in good colocalization with GFAP, MnSOD and GS stains and with literature [1, 4]. It was found that astroglial cells highly immunostained for GFAP would secrete reactive oxygen species, resulting in an upregulation of MnSOD and GS [2, 10], which are Mn dependent enzymes. In addition, glutamate could be released at the peri-infarct areas by increasing the activation of neuronal

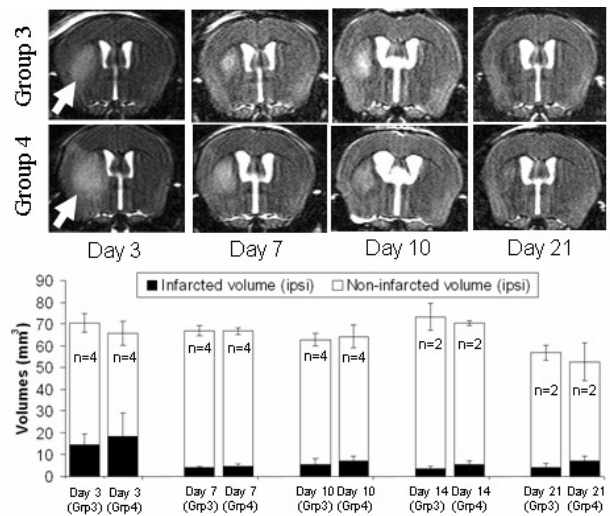


Fig. 5. (Top) Typical T2WI at Bregma -0.58 mm, showing hyperintensity in the ipsilesional dorsolateral striatum (arrows) with (Group 3) and without (Group 4) Mn^{2+} injection. (Bottom) Total infarct volumes indicated by T2W hyperintensity between Bregma -1.70 mm and 1.54 mm in the ipsilesional forebrain were not different between the groups (unpaired t-test, $p>0.05$).

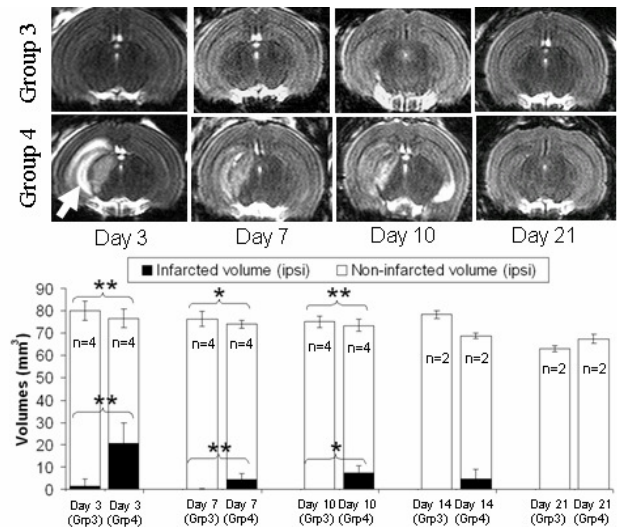


Fig. 6. (Top) Typical T2WI at Bregma -3.08, showing hyperintensity in the ipsilesional midbrain and hippocampus (arrow) in Group 4 but not Group 3. (Bottom) Total ipsilesional infarct volumes in the posterior part of the brain between Bregma -4.96 mm and -1.70 mm had significantly reduced after Mn^{2+} injection (unpaired t-tests between two groups on infarct volumes (black) or non-infarct volumes (white), ** $p<0.01$, * $p<0.05$).

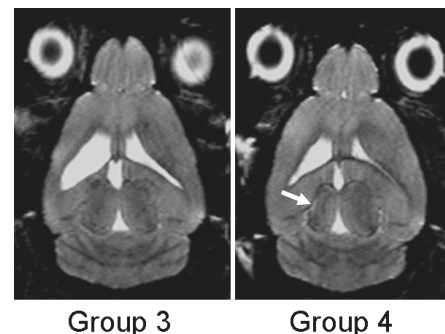


Fig. 7. Axial T2WI of mouse brains at 150 days after MCAO. An apparent shrinkage of the ipsilesional midbrain (arrow) was observed in all the 2 animals remained in Group 4 compared to Group 3.

glutamate receptors which in turn increased membrane permeability to Ca^{2+} in astrocytes [11-13]. Given Mn^{2+} has been demonstrated as a calcium analog [14], it is possible that exogenous Mn^{2+} injection would lead to enhanced MEMRI detection of oxidative stress and gliosis [2, 5]. Note that hyperintense signal areas in MEMRI were shown to match perfectly with areas of microglial activation in the transgenic mouse brains at terminal disease stage [15].

On the other hand, it has been recognized that brain regions remote to the striatum were less susceptible to oxidative stress [16] and might undergo later onsets for delayed neuronal death due to ischemia and reperfusion [17]. Microglial reaction at remote sites such as the neocortex, thalamus and both hippocampi took about 5 days to become fully apparent after transient focal cerebral ischemia [18], whereas brain macrophages were clearly visible at 3-7 days postischemia [19]. Previous MEMRI studies have shown that Mn^{2+} accumulated and gave peak enhancements in T1WI in the posterior parts of the mouse brain within 24 hours after systemic Mn^{2+} administration [20]. Given that Mn^{2+} triggered the scavenging of superoxide and hydroxyl radicals [5], whereby MnSOD and GS activities increased upon MnCl_2 administration to normal and ischemic animals [2, 5, 6], it is possible that the reduction in infarct volumes in T2WI in the posterior parts of the MCAO mice might be associated with neuroprotection of Mn^{2+} at remote sites before apparent delayed secondary damage took place, though further analyses are needed to address this issue. Note also the less neuronal shrinkage in the midbrain areas of the mice in all the 2 remaining animals in Group 3 when compared to those in Group 4 at 5 months after MCAO in Figure 7. Recent studies have shown marked neuroprotective effects of manganese complexes against focal ischemic insults up to 6 hours after ischemia [21, 22]. The results of this study illustrated the potential neuroprotective effects of manganese after administration at a longer postischemic delay. No significant difference was observed in the infarct areas in the forebrain of MCAO mice or the PCI rats between groups with and without Mn^{2+} injection, possibly due to the ischemic core being readily damaged before Mn^{2+} administration.

V. CONCLUSIONS

Given the distribution of T1W hyperintensity was similar to that of MnSOD and GS immunoreactivities reported [2, 4], it is likely that exogenous Mn^{2+} injection may provide enhanced MEMRI detection of oxidative stress and gliosis early in the PCI and MCAO models of different strains. Manganese may also mediate remote brain infarctions in transient focal cerebral ischemia before delayed secondary damage takes place.

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